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Morphology and Distribution of the Skin Glands in *Xenopus laevis* and Their Response to Experimental Stimulations

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ABSTRACT—Four types of skin glands were observed in the skin of adult *Xenopus laevis*. Among them, the mucous and the granulated glands showed similar morphological characteristics with those previously reported. In this report, we made detailed description on the saccular gland (referred to as NP gland in the present study) of the male nuptial pad. The small granulated gland which has not been reported so far is also described. The granulated gland and the NP gland were observed to receive innervation inside the secretory compartments. The NP glands were only distributed in the nuptial pad of the male forelimb while the other three kinds of glands were widely distributed throughout the body surface. When the frogs were kept under waterless conditions, the mucous glands secreted a watery substance, the cross sectional area of the skin occupied by the mucous glands being reduced to 46–71% of the control. While, the granulated gland and the small granulated gland were less affected by the water deprivation. Evacuation of contents of the skin glands was recognized only in the mucous glands. After hypophysectomy, a significant decrease in the area of the NP glands occurred accompanied by the disappearance of the nuptial pad, while the mucous and the granulated glands were unchanged. A possible enhanced secretory activity of the small granulated glands was encountered after hypophysectomy. Isoproterenol induced the discharge of viscid white material from the contracted granulated gland both *in vivo* and *in vitro*, while other types of skin of glands seemed to be unchanged by this chemical.

INTRODUCTION

The skin glands of amphibians have been classified into major types, *viz.*, the granulated glands and the mucous glands, based mainly on light microscopic observations [1, 2]. The granulated glands are involved in protecting the body through their poisonous or irritating secretions in response to violent stimulation [3], while the mucous glands produce a watery secretion containing Na^+ and Cl^- that keeps the skin moist and controls its permeability [4]. Among the skin glands of *Xenopus laevis*, the granulated gland has been intensely investigated mainly due to interest in the biochemistry of its secretion. The granulated gland of *X. laevis* has been reported to contain thyrotrophin releasing hormone, 5-hydroxytryptamine [5] and caerulein [6, 7]. Dockray and Hopkins [6] also reported that the dermal layer of the dorsal skin of *X. laevis* contains several kinds of glandular structures, but there was no description of gland other than the granulated and mucous glands.

During the course of the investigation of the nuptial pad of male *X. laevis* [8], two other types of skin glands could be differentiated. Berk [9] briefly described one of them as a simple saccular gland in the nuptial pad, and the other has not been possibly identified in any previous report cited. In the present paper we provide a classification of different types of skin glands of adult *X. laevis* after studying of fourteen regions all over the body surface of both sexes accompanied with a study of changes in these glands after exposing frogs to a waterless condition, hypophysectomy

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and adrenergic stimulation. The possible relationship of these glands to the internal and environmental modifications is also discussed.

MATERIALS AND METHODS

Animals

The African clawed frogs *X. laevis* which were successively kept in our laboratory were used.

Light and electron microscopy

Excised skin pieces from frogs, after decapitation and pithing or under 0.5% ethyl m-aminobenzoate methanesulfonate anesthesia, were fixed with Bouin's fluid, processed routinely for light microscopy and stained with Mallory's trichrome. To study on the distribution of skin glands through the body surface, pieces of skin of about 2 cm square were excised from fourteen selected areas from both sexes as shown in Figure 3. To study the ultrastructure, skin pieces were fixed in 3% glutaraldehyde followed by postfixation with 1% osmium tetroxide and embedded in an Epon-Araldite mixture. The prepared ultra-thin sections were observed with Hitachi HS-8 and LEM 2000 electron microscopes.

Response of the skin glands to various experimental conditions

Dry milieu Adult frogs of various body weights were housed in separate containers without water (75–80% humidity and 21±0.5°C). Body weight was recorded every 24 hr exactly. At later stages of the experiment, pieces of skin in the central regions of ventral and dorsal sides were dissected out and processed for histological observation.

Hypophysectomy Adult males with well-developed nuptial pads were hypophysectomized. Frogs then were observed for macroscopic changes in their nuptial pads. Representative frogs were sacrificed on 12 and 40 days and at 15 months after the operation for histological observation. All quantitative measurements of the area, length and number of glands and of skin were carried out with an image analyzing apparatus (VIP, 121CH, Olympus Co., Japan).

Sympathomimetic stimulation The response of skin glands to isoproterenol was tested *in vivo* and *in vitro* as follows.

Experiment 1; For the *in vivo* study, 2 ml of isoproterenol (ISP) (Sigma) at a concentration of 2×10^{-2} M/100 g body weight was subcutaneously injected into the adult frogs.

Experiment 2; An *in vivo* study concerning the interruption of vascularization and innervation of skin pieces was done as follows. Pieces of dorsal skin about 1.5×1.0 cm in size were first cut completely free from their surroundings, and were then stitched into the same position as before. Seven days after the operations, the frogs were subcutaneously injected with ISP and their responses were observed.

Experiment 3; For *in vitro* study explants about 2 cm square were taken from the middle of the dorsal or ventral sides and were immersed in Ringer solution or TCM 199 solution for up to 14 days. Response of the freshly excised skin to ISP was first tested employing the solutions containing ISP in concentrations of 10^{-1} to 10^{-5} M. The pieces of skin incubated were transferred every other day to the solution containing 10^{-1}M ISP to examine their reactivity.

Noradrenaline (Sigma) was also used *in vivo* and *in vitro* study to test the reaction of the skin glands.

RESULTS

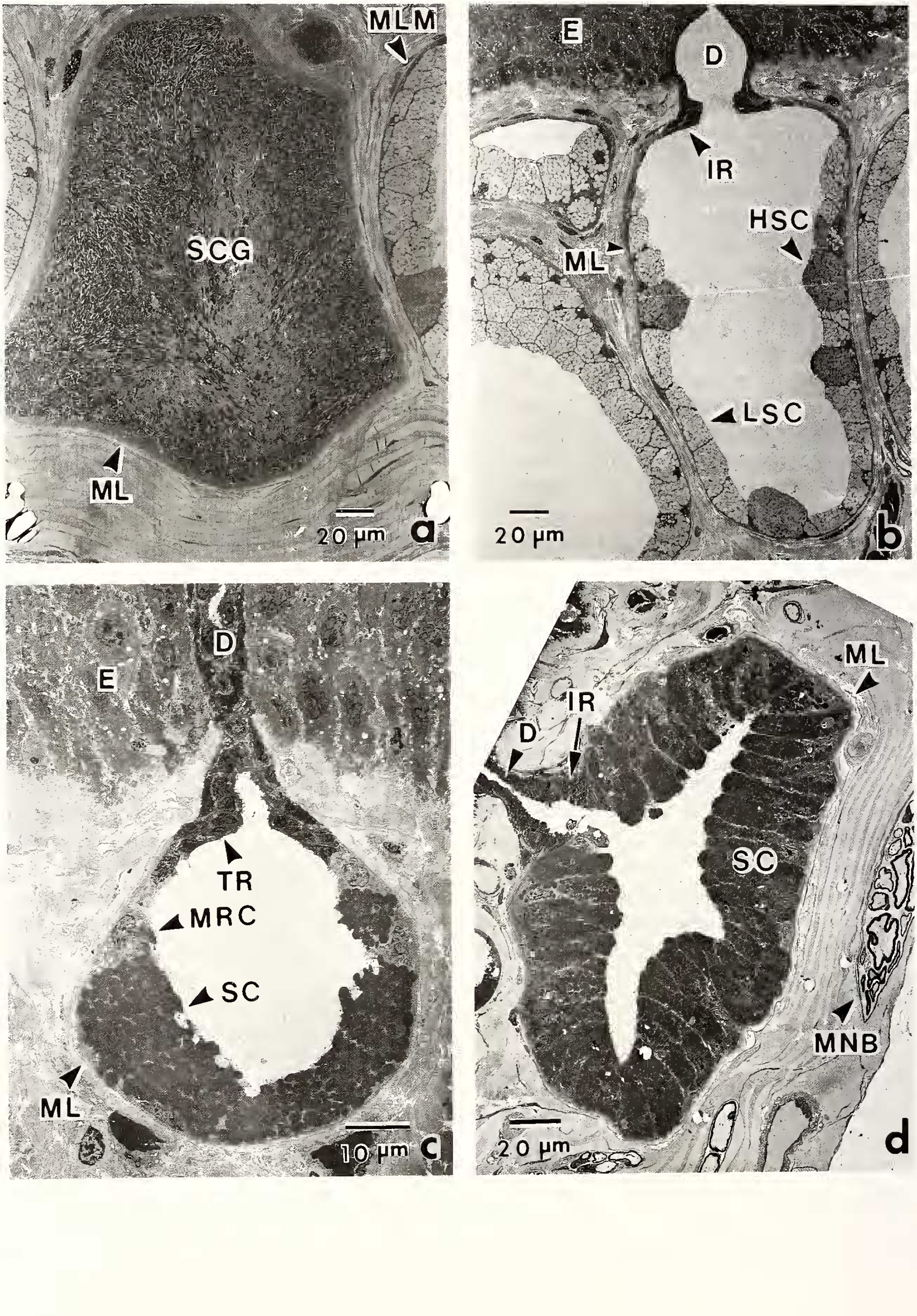
Morphological characteristics of the classified skin glands

Through the microanatomical observations of the skin from several regions of the body surface, the following four types of skin glands were identified.

The granulated gland The granulated gland was composed of a secretory compartment, a duct and an intermediated region connecting them. This type of gland was largest of the four types of glands observed, ranging 150 μm up to 200 μm in height and 200 to 400 μm in diameter. Above all the dorsal side of a large-sized female observed had a group of the largest granulated glands, measuring from 350 to 450 μm in height and from 300 to 550 μm in diameter. The secretory compartment consists of a syncytial sac surrounded by a myoepithelial cell layer (Fig. 1a). In a histological view of a gland which might be a median profile of a secretory compartment, there were observed twelve peripherally arranged nuclei profiles with their long axes parallel to the myoepithelial layer. The electron microscopic (EM) observations revealed that the secretory granules which occupy the greater part of the syncytial cell were elipsoidal in shape, measuring $2.7 \times 0.8\,\mu$m in average. These secretory granules were membrane-bounded and varied in electron density. Their contents were heterogeneous showing electron dense stripes randomly distributed and running parallel to the long axes of the granules (Fig. 7a, b, c). In the cytoplasm, well-developed rough ER and Golgi apparatus were observed surrounding the nucleus. In the area of the Golgi apparatus small dense granules were prominent, some of them appeared to be larger and membrane-bounded containing homogeneous substance, which might be a sign of the formation of secretory granules.

In the intermediate region between the secretory compartment and the excretory duct, two different types of small cells were observed. The cells located at the inner side, i.e. near the compartment, have a central large nucleus and distinct microvilli projecting into the ductal lumen with no secretory granules. On the other hand, the outer side cells were larger than the inner ones, interdigitated with each other and continued to the ductal cells. The excretory ducts of the glands were opened throughout its length onto the surface of the skin, and some secretory materials were occasionally found to remain in the ductal lumen. The duct was a bilayered structure whose cells are columnar, the outer cells being a little slenderer than the inner ones. The myoepithelial cells which completely enwrap the secretory compartment are cuboidal in cross section and their width and height were about 7–9 μm and 4–6 μm, respectively. The individual myoepithelial cells in longitudinal sections were found to face each other with indented cellular surface on which desmosomes were frequently seen. Thick myofilaments were distributed equally through the cytoplasm and the smooth ER were well developed in the peripheral cytoplasm facing the secretory cells (Fig. 7a) or adjacent to the myoepithelial cells. Numerous pinocytotic vesicles were found along the plasma membrane of the myoepithelial cells, especially facing the secretory cell cytoplasm. Irregular finger-like projections from the secretory cells came close to the myoepithelial cells and desmosomes were found in these areas, especially in contracted cells (Fig. 7b). Extreme contractions of the myoepithelial layers of the granulated gland were always present in the biopsied specimens whose secretory products were expelled via the excretory ducts. Both myelinated and non-myelinated nerve bundles, capillaries and melanocytes were found in the subcutaneous connective tissue around the gland. In the vicinity of the granulated gland, nerve bundles were divided into small nerve branches and the non-myelinated nerve bundles were seen close to the glands. It was observed that these non-myelinated fibers run throught the intercellular spaces between the adjacent myoepithelial cells and are distributed in the intercellular spaces between the myoepithelial cells and the secretory cells with a number of nerve ending structures on the surfaces of the myoepithelial cells or of the secretory cells (Fig. 2a). Occasionally these nerve endings were found to rest on the deeply concave surfaces of the secretory cells. No partial thickening like pre- or postsynaptic membrane could be found in these areas. Individual nerve endings were seen to contain a large number of small vesicles of the size of synaptic vesicles (about 50–60 nm), a few cored vesicles (about 100–150 nm) and mitochondria. The serially arranged pinocytotic vesicles along the surfaces of myoepithelial cells were frequently opened toward the intercellular spaces at places close to nerve ending structures.

The mucous gland The mucous gland was composed of a secretory compartment, a duct and an intermediate region between them (Fig. 1b). The glands of this type in the male under normal conditions measured approximately 125 μm in height and 184 μm in width. In cross sectional profile the secretory cells were found to be arranged in a monolayered or a multilayered pattern along the periphery of the secretory compartment, and in horizontal section they appeared somewhat pentagonal in shape. Most of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them were stained light blue with Mallory's triple stain and some were stained dense blue. With the EM, the structural cells of a compartment could be classified into two types, one appearing as a low density cell possessing granules of low electron density and the other a high density cell with electron-dense granules (Fig. 1b). The latter type had a strongly positive periodic acid-Schiff (PAS) reaction. The first type of secretory cells was found to be the prevailing type while the second type was less represented, forming only about one quarter of the total number of secretory cells in a compartment. With the EM, the clear granules were occasionally seen to be vacuolated square, pentagonal or round in shape measuring 2.4 μm along the longer axis and 1.7 μm along the shorter axis in average (Fig. 2b). Dark granules were not uniform, were of varied deformed eggplant shapes and contained irregular filamentous deposits that run roughly parallel to the long axis of the granules. They occasionally fused with each other. Numerous dark spots were also present in the granules. The nuclei of cells were situated basally and surrounded by a moderate number of rough ER. Many interdigitations and occasional desmosomes were found in the intercellular space between the neighbouring secretory cells. Toward the basal region of the excretory duct, the cell type in the secretory compartment changed abruptly. In this area the cells adjacent to the secretory cells were slender and elongated containing a moderate number of mitochondria with no secretory granules. This cell type was adjoined to a cell aggregation forming the base of the excretory duct (Fig. 1b). In this cell aggregation, the inner side cells which were adjoined to the ductal cells were small and columnar in shape. They possessed large nuclei and were provided with numerous microvilli. The outer side cells were elongated and almost the same size as the inner side cells. Well-developed interdigitations were observed between the inner and the outer side cells, occasionally with demosomes. These cells were adjoined to the bilayered ductal cells of which the inner side cells were of small and columnar shape. The outer side cells were slightly larger than the inner side cells. Cornified ductal cells were continuation of the epidermal cornified layer. The myoepithelial cells surrounding the mucous gland were flat and elongated and not so thick as those of the granulated gland (Figs. 1b and 2a), measuring 0.6 to 0.8 μm in thickness which is about one tenth of that of granulated gland. The intercellular space between the secretory cells and the myoepithelial cells was of a simple appearance as compared with that of the granulated gland. Both myelinated and the non-myelinated nerve bundles were seen in the dermal connective tissue around the mucous glands (Fig. 2b). However, they did not pass through the intercellular space between the adjacent myoepithelial cells.

The small granulated gland The small granulated gland was also composed of a secretory compartment, a duct and an intermediate region between them (Fig. 1c). This gland has not been described in the literature cited so far. This type of gland was located close to the epidermal layer of the skin and was the smallest among the four types of skin glands observed in the present study. It averages 50 μm in height and 60 μm in width. The nuclei, rough ER and Golgi apparatus were confined to the base with a large amount of secretory granules occupying most of the cytoplasmic area. The secretory granules were of a uniform appearance and they became yellow with Mallory's stain. With the EM, the granules were

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Fig. 1. Electron micrographs of four types of skin glands.

a: A granulated gland showing the syncytial appearance of a secretory compartment and thick myoepithelial cell layer. On the right hand side of the figure, a thin myoepithelial cell layer of a mucous gland can be seen. SCG, secretory compartment of granulated gland; ML, muscle cell layer of granulated gland; MLM, muscle cell layer of a mucous gland.

b: Mucous glands showing low density secretory cell (LSC) and high density secretory cell (HSC) intermediate region (IR) and excretory duct (D). E, epidermis; ML, myoepithelial cell layer.

c: A small granulated gland showing secretory cell (SC), intermediate region with mitochondria-rich cells (MRC) and transition region (TR). D, secretory duct; ML, myoepithelial cell layer; E. epidermis.

d: A NP gland showing secretory cells (SC), secretory duct (D) and intermediate region (IR). ML, myoepithelial cell layer; MNB, myelinated nerve bundle.

found to be electron-dense and spheroidal in shape with an average size of 1.9×3.2 μm, including the largest one that measured around 6 μm in diameter (Fig. 1c). This is the largest among the secretory granules of the four kinds of skin glands observed. In the intermediate region adjacent to the secretory cells, mitochondria-rich cells were observed adjoining the transition region of the excretory duct (Fig. 2c). Other than numerous mitochondria, these cells were characterized by their microvilli, large round nuclei and the cytoplasm free of secretory granules. The arrangement of cells at the transition region toward the excretory duct and that at the excretory duct were the same as those of the mucous gland. The myoepithelial cells discontinuously surrounded the secretory compartment. The myoepithelial cells of the small granulated gland were wider than those of the mucous gland, but narrower than those of the granulated gland. The myelinated and non-myelinated nerve bundles were seen to have access to the gland, and the nervous structures which seemed endings or near the endings containing number of small vesicles and some cored vesicles, were occasionally observed in the area close to the basement lamina of the myoepithelial cells (Fig. 2c). However, no innervation was found in the intercellular space between the myoepithelial cells and the secretory cells.

NP gland NP glands were found only in the nuptial pad. The average size of the gland was 160 μm in width and 99 μm in height in a frog weighing about 43 g. This gland consists of a secretory compartment, a duct and an intermedi-

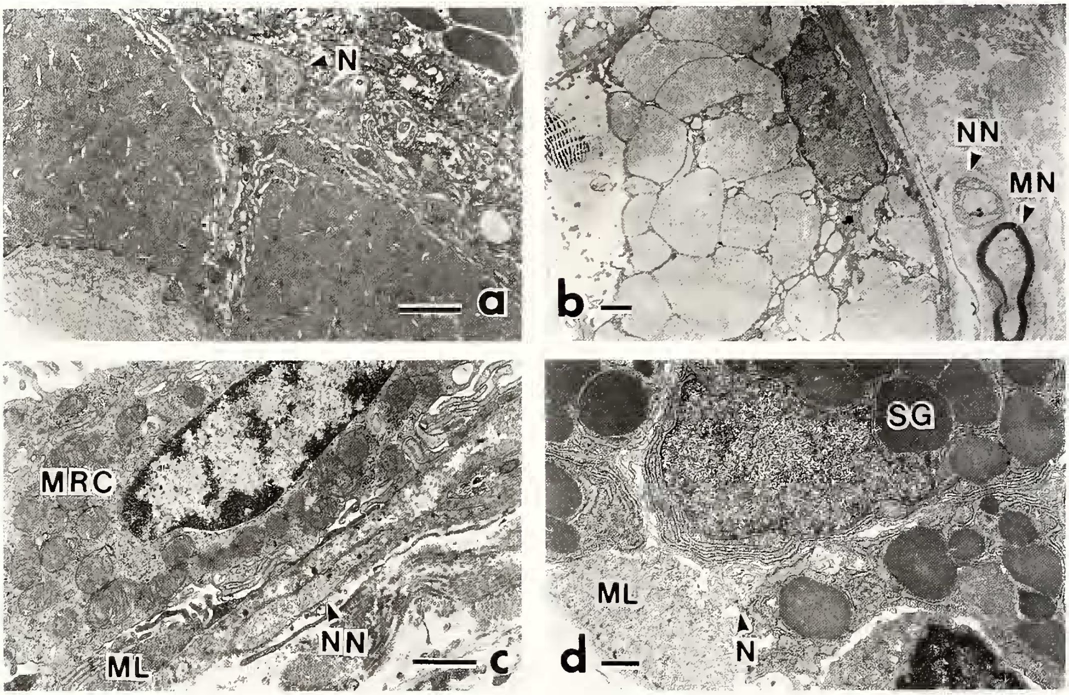


Fig. 2. Electron micrographs showing innervations in four types of skin glands.

a: A nerve ending structure in the intercellular space between myoepithelial cell and secretory cell of a granulated gland. N, nerve ending.

b: A profile of a mucous gland showing a secretory cell with clear vesicles, and a non-myelinated nerve bundle and a myelinated nerve with access to the secretory compartment. MN, myelinated nerve, NN, non-myelinated nerve.

c: A small granulated gland showing a mitochondria-rich cell in a secretory compartment and non-myelinated nerves which face a thin myoepithelial cell layer (ML). MRC, mitochondria-rich cell.

d: A NP gland showing secretory cells with large secretory granules (SG), a myoepithelial cell in longitudinal section (ML), and a nerve ending (N) surrounded by secretory cells.

ate region between them (Fig. 1d). The secretory compartment was composed of monolayered columnar secretory cells which were arranged in the bottom of the compartment. The average size of secretory cells was 38 μm in height and 7 μm in width. The secretory cells lodged with a large quantity of secretory granules and their nuclei were situated at the base. These granules were stained deep blue by Mallory's triple stain, and yielded a strong positive reaction to PAS. With the EM, profiles of well developed layered rough ER and Golgi apparatus were seen near the nucleus, and the apical part of the cells was provided with numbers of microvilli protruding into the lumen of the compartment. The adjacent cells were interdigitated with each other with occasional desmosomes. In a profile of a secretory cell, there were observed 50 to 60 membrane-bounded granules which were homogeneous and mostly spheroidal in shape. In the areas of Golgi apparatus there were occasionally observed small granules of varied electron density, measuring 1.8×1.2 μm in average, which seemed to be in a process of maturation. Some of these small granules were in shapes suggesting that secretory granules might also become larger by the fusion of small granules together. The glands were surrounded discontinuously with myoepithelial cells which were moderately developed as in the case of the small granulated gland. In the regions where

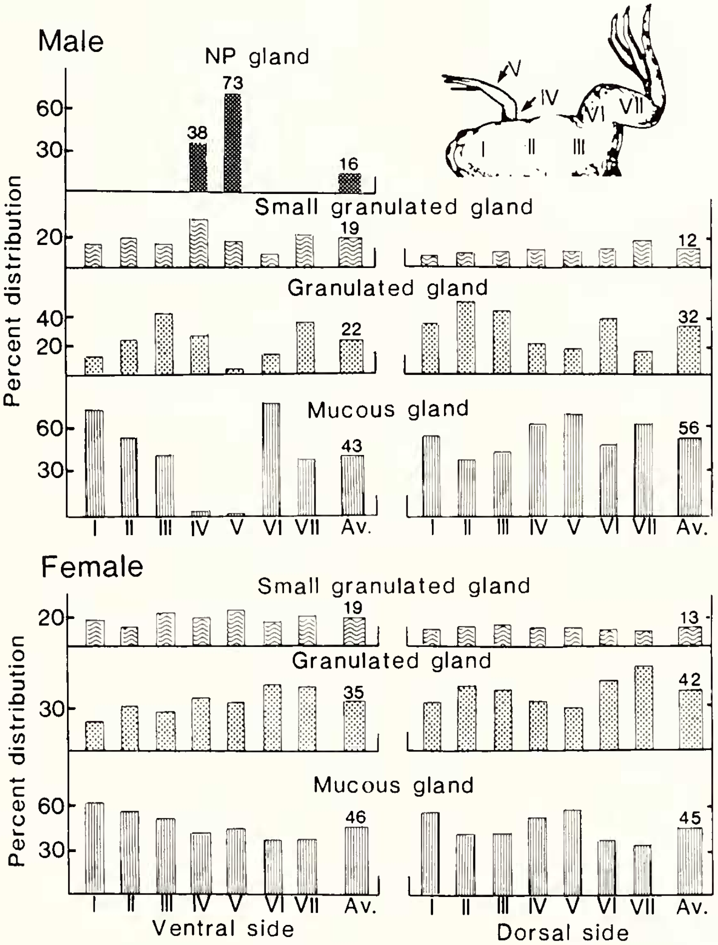


FIG. 3. Percent distribution of skin glands in selected regions of body surfaces. I–VII indicate areas selected for observations as shown in the right hand corner.

the secretory cells were not covered with myoepithelial cells, the basement lamina of the secretory cells directly faced the connective tissue surrounding the glands. In the connective tissue surrounding the NP glands, there were observed intense vascularizations. The terminal structures of the non-myelinated nerves were observed to rest on the surfaces of the secretory cells or the myoepithelial cells after passing through the intercellular space between the adjacent myoepithelial cells (Fig. 2d). Also the terminal structures were observed to make direct contact with the secretory cells at the place where the secretory cells had no myoepithelial cell covering.

Distribution of skin glands through the body surfaces The distribution of four types of skin glands in selected areas of the body surface of both male and female frogs is shown in Figure 3. Distribution is shown as the number of each skin gland as a percentage of the total number of skin glands counted in the area selected for study. In the male, the percentages of mucous glands, granulated glands, small granulated glands and NP glands were 43, 22, 19 and 16% on the ventral side and 56, 32 and 12% on the dorsal side, respectively, while in the female they were 46, 35 and 19% on the ventral side and 45, 42 and 13% on the dorsal side, respectively. The percentage distribution of NP glands was 73% at V and 38% at IV in the male. In the nuptial pad region occurrence of mucous glands and the granulated glands was infrequent. No NP glands were found in other regions.

Changes in skin glands under the experimental conditions

1) Dry milieu

Figure 4 shows the decrease in body weight after keeping in a waterless condition. The percent reduction in body weight varied among the experimental frogs and the small-sized frogs were apt to show a larger reduction (32–34%) than the larger ones (21–34%). The skin pieces excised from the experimented frogs as well as the controls were subjected to histological studies to measure the heights of the skin and epidermis, sectional areas of the mucous glands, granulated glands and small granulated glands. In the control frogs, all these measurements in the females were larger than those in the males (Table 1). It is worth noting here that the area of the granulated glands on the dorsal side was about twice as great as that of those on the ventral side. When the frogs were kept in a waterless milieu for 3 days, all the measurements in the skin and the skin gland became smaller than those of the controls. The thickness of the skin became 73–83% of that of the controls, the epidermis 63–82%, and the areas of the mucous gland 29–54%, the granulated gland 63–80% and the small granulated gland 74–98%.

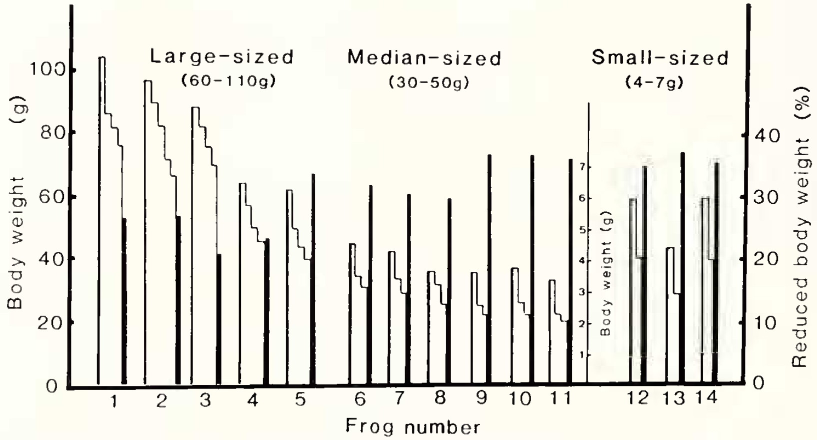


Fig. 4. Daily changes in body weight after keeping the animals under waterless conditions. Dark bars indicate reduced body weight as a percentage reduction as determined by final weighing. ( ) indicates body weight of frogs.

TABLE 1. Measurement of skin and skin glands of *Xenopus laevis* kept in water (normal) and under waterless conditions

	Male					Female				
	Height of skin in μm		Areas of skin glands in μm^2			Height of skin in μm		Areas of skin glands in μm^2		
	Skin	Epi-dermis	Mucous	Granulated	Small granulated	Skin	Epi-dermis	Mucous	Granulated	Small granulated
					Ventral side					
Normal	372±4[†] (25)	56±1 (24)	10,999±433 (72)	25,125±953 (52)	1,743±206 (22)	568±7 (24)	110±2 (22)	22,741±1,506 (61)	76,821±4,151 (39)	3,221±579 (24)
Waterless	289±6* (25)	35±1* (24)	4,079±222* (72)	19,652±1,374* (51)	1,299±126 (23)	473±6* (24)	90±2* (21)	6,487±379* (59)	60,612±3,034* (49)	3,164±339 (19)
					Dorsal side					
Normal	544±4 (24)	55±1 (24)	17,304±876 (56)	55,935±3,122 (60)	1,670±99 (23)	645±7 (24)	66±1 (23)	19,247±1,103 (56)	123,929±6,803 (58)	1,809±229 (20)
Waterless	397±5* (25)	36±1* (23)	9,305±585* (73)	44,835±2,629* (62)	1,484±178 (19)	520±6* (23)	55±2* (21)	8,418±637* (57)	77,811±4,885* (57)	1,515±126 (24)

[†] Mean±S.E.

( ): Number observed.

* Significant as compared with the control ($P<0.01$, with t-test).

The present study revealed that the area of the mucous gland showed the most remarkable reduction, especially in those on the ventral side (down to 37% in the male and 29% in the female). The sectional areas of their glands were reduced, and the evacuation of contents of the glands was also recognized histologically. In addition, low density cells in the compartment of the atrophied mucous gland seemed to be reduced in size more than high density cells. The granulated glands seemed to be unchanged.

2) Hypophysectomy

A significant decrease in the area of the NP gland occurred after hypophysectomy and the size of gland became about half that of the unoperated control frog, while the mucous, the granulated and the small granulated glands were unchanged (Table 2, Fig. 5a, b). On day 12 after hypophysectomy, the NP gland cell already showed a decrease in diameter, area and height (Table 3). Such regressed features were persisted in the specimens kept for 15 months after hypophysectomy (Fig. 5c). The area of the secretory cell nuclei was unchanged. The amount of secretory granules was also decreased on day 12 and became much less on day 40 without recovery later. In association with regression of the NP gland, the numerous small epidermal spikes which specifically cover the nuptial pad area were rapidly decreased in number followed by complete disappearance in the 40 day specimens. There were often seen profiles of the small granulated gland which seemed to show active secretion through their distended excretory ducts which contained a considerable amount of secretory material. Such a histological view was unusual in other experimental series as well as in the controls.

3) Isoproterenol (ISP) application

Experiment 1: Within a minute after the injection of ISP, the recipient frogs started to react to ISP and in about 5 min the entire body surface was covered with milky and viscid secretory material expelled from the granulated glands. Histologically, evacuation of the contents of the glands occurred only in the granulated glands in which the myoepithelial layer showed remarkable undulation accompanied by hypertrophy in some places. The mucous gland and the small granulated gland were

Table 2. Effect of hypophysectomy on the areas of skin glands 40 days after the operation

Skin glands	Area of gland in μm^2 (Mean±S.E.)	
	Control	Hypophysectomy
Mucous	11,002±1,211 (71)	10,833±1,195 (61)
Granulated	15,686±2,089 (25)	13,352±1,559 (26)
Small granulated	2,794±441 (22)	2,975±233 (21)
NP	9,779±1,766 (9)	4,867±412 (6)*

( ), number of glands observed.
* $P<0.05$ with t-test.

Table 3. Morphological alterations in NP glands after hypophysectomy

Days after operation	Number of glands observed	Diameter (μm, Mean±S.E.)		Cross sectional areas (μm^2, Mean±S.E.)			Height$^+$ of gland cell (μm)	Area$^+$ of nucleus (μm^2)
		long	short	Gland (a)	Lumen in compartment (b)	(a)–(b)		
0	23	99±2	160±6	12,549±881	1,877±216	10,671±718	30–55	25–34
12 days	28	84±3**	138±5	9,194±567*	2,715±359	6,478±409**	20–35	23–29
40	23	56±2**	130±7**	4,847±338*	1,478±284	3,369±290**	10–20	23–27
15 months	23	69±2**	116±5**	5,783±362**	3,407±355**	2,376±117**	5–20	26–32

* $P<0.05$, ** $P<0.01$, with t-test.
+ shows only ranges of measurements.

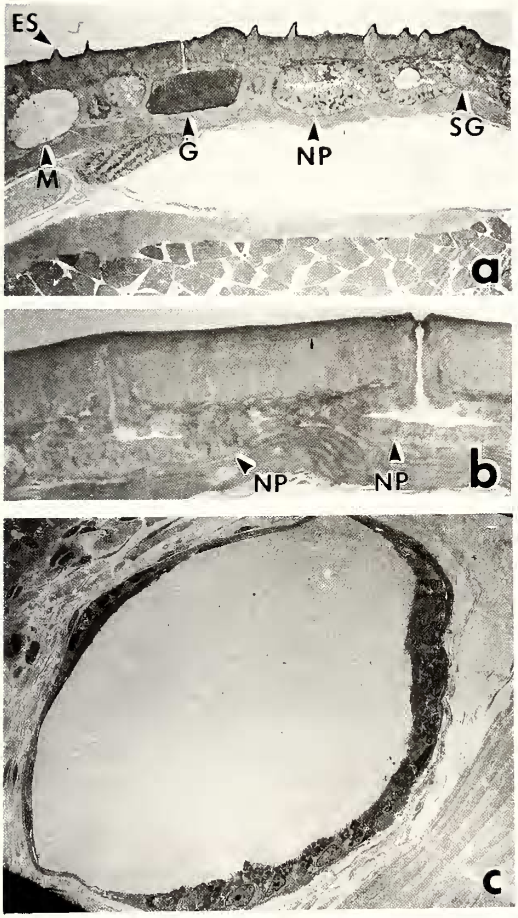


FIG. 5. Histological views of nuptial pads showing a control (a), and operated animals in 40 days (b) and 15 months (c) after hypophysectomy. ×53 (a), ×145 (b), ×470 (c). ES, epidermal spike; NP, NP gland; M, mucous gland; G, granulated gland; SG, small granulated gland.

unchanged (Fig. 6b).

Experiment 2: Upon injection of ISP, the operated skin areas on the backs of the frogs did not respond to a drug injected although the surrounding intact skin responded well in a way similar to that of normal frogs (Fig. 8). The operated skin area showed gradual response to injected ISP with time, following operation days. The central area of the operated skin pieces started to react weakly to the injected ISP by post-operative day 16. Approximately a half area of the operated skin piece was observed to respond to ISP on day 28.

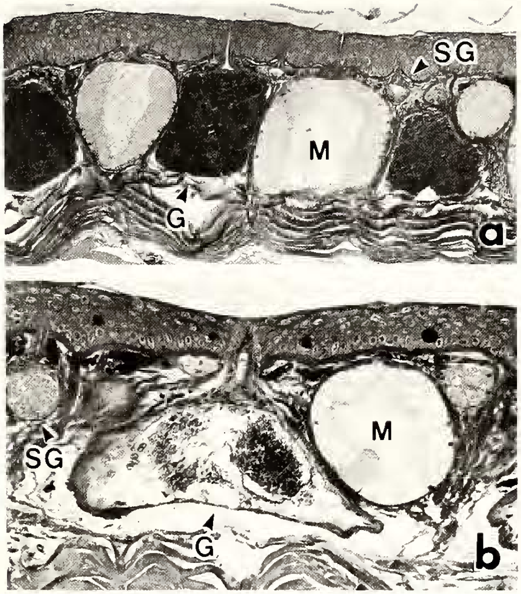


FIG. 6. Histological views of skin glands of a control skin (a) and of a skin affected 30 min after ISP injection (b). ×110 (a), ×110 (b). G, granulated gland; M, mucous gland; SG, small granulated gland.

Experiment 3: The skin pieces taken from the body surfaces showed results similar to that in the Experiment 2 when they were immersed in the physiological saline or medium containing ISP in concentrations ranging from 10^{-1} to 10^{-5} M. With the EM, 30 min after immersion in ISP solution (10^{-1} M), the intercellular space between the secretory cells and the myoepithelial cells was seen to be wider, and a large number of vascular structures appeared at the periphery of the secretory cells (Fig. 7d). Secretory granules were sparsely distributed (Fig. 7c). The fibrillar sarcoplasm of the myoepithelial cells had a condensed appearance. However, in the sarcoplasm facing the basal lamina of the secretory compartment, a number of small protrusions appeared in the specimens fixed 5 sec after ISP immersion (Fig. 7b). The higher the concentration of ISP applied the more rigorous was the response of the glands to it. The lower the concentration of ISP, the more time needed for the glands to respond and the weaker was their response. At a concentration of 10^{-5} M the reaction was obscure. These skin

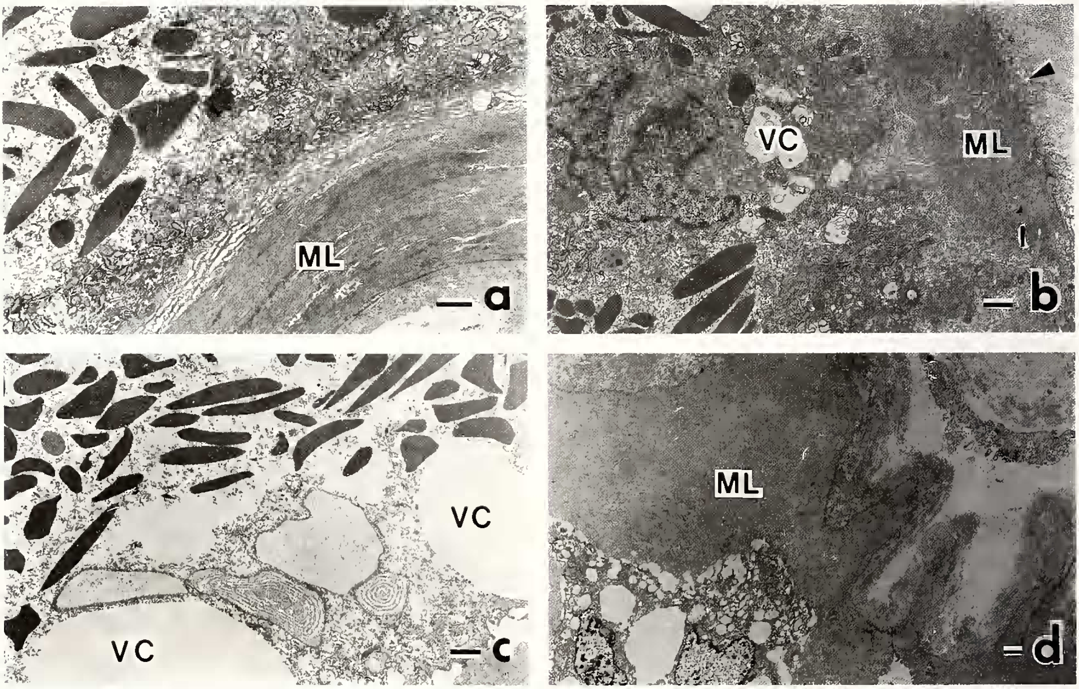


Fig. 7. Electron micrographs of granulated glands of a control (a), and 5 sec (b) and 30 min (c and d) after immersion in ISP solution. Arrow shows small sarcoplasmic protrusions. ML, myoepithelial cell layer; VC, vascular formations.

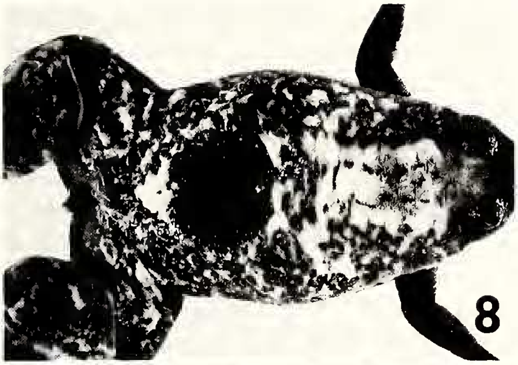
pieces were able to respond to ISP even after they were kept for as long as 14 days (Fig. 9). However, in this case the response occurred sporadically and not through the entire skin surface.

In vivo and *in vitro* application of noradrenaline at a concentration of 10^{-5} M was able to induce a discharge of secretion.

DISCUSSION

Classification of skin glands

The granulated and mucous glands have been reported to be the major types of the amphibian skin glands [1]. Mills and Prum [10] classified the mucous glands of *Rana pipiens, R. temporaria* and *R. catesbeiana* as mucous and seromucous glands on the basis of electron microscopic observations. According to this report the mucous gland has mitochondria-rich cells in the junctional area between the duct and the acinus while the seromucous gland has cells without granule in the same region. Fox [11] introduced kinds of amphibian skin glands as poisonous, lumpy, callous, mucous, and so on. In *Salamandrina terdigitata,* three types of skin glands, that is the mucous, serous and the mixed type, have been described [12]. These indicate that the skin glands of amphibians vary in form, and the terminology for these glands has not been well established. In the case of the skin glands of *X. laevis,* most of the studies have been concentrated on the granulated gland due to the interesting biochemical nature of its secretion and a little has been elucidated about other type of skin glands, although it was reported that the dorsal skin of *X. laevis* contained several kinds of grandular structures opening via the epidermal ducts to the external surface [6]. In the present study, we were able to demonstrate four different types of skin glands in the body surface of adult *X. laevis.* These were the mucous, the granulated, the small granulated and the NP glands. It appeared that the small granulated gland and the NP gland as termed in the present report were different



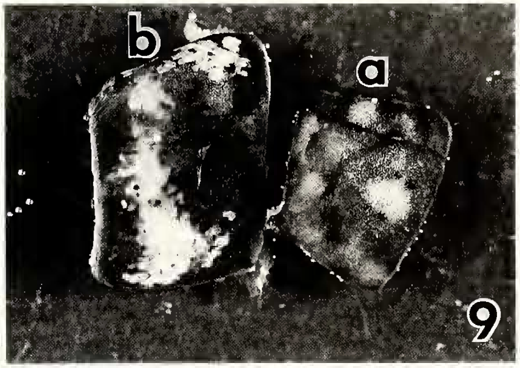


FIG. 8. Response of skin granulated glands about 5 min after the injection of ISP. No response is seen in the operated skin (Experiment 2) at the center of the back, 7 days post-operation. ×0.55.

FIG. 9. Response of the *in vitro* granulated gland kept in medium 199 (a) and in physiological saline (b) for 12 days followed by immersing them in the media containing 10^{-1} M ISP for 5 min. ×2.

types from the seromucous or mixed type which was described previously in other species, as well as from the mucous and the granulated glands of *X. laevis* in their morphology, and responses of glands to various types of stimulation will be mentioned later.

Distribution of skin glands

Except NP gland the other three types of skin glands are distributed throughout the body surface. The mucous gland and the granulated gland were found to be greater in number and larger in size. This is in agreement with a report by Sjöberg and Flock [13] on *R. temporaria* and *R. esculenta.* However, the NP gland was found to be distributed only in the male nuptial pads. According to Berk [9], in the nuptial pad area there were only glands of a simple saccular type (NP gland) and no other glands were found in this area. However, in the present study it was found that the granulated and the mucous glands existed in the nuptial pad area although a few in number. Also quite a number of small granulated glands were observed in this area.

Stimulation of glands

The response of the mucous and the granulated glands to adrenergic agonists observed might be similar to that of the previous reports [4, 14, 15]. However, to what kind of stimulation the small granulated gland responds could not be clarified by the present experiment. We only found that some of the small granulated glands expelled much of their contents into the excretory ducts when the frogs were hypophysectomized. Many investigators have reported that some kinds of glands were present underneath the papillae in thumb pads of male frogs and newts [16–18]. However it has been still not certain whether skin glands in the area of thumb pads are the same or different from those of the common skin glands. As has already been reported, the development of the pads was dependent on the androgenic hormonal cycle [16–18], and the development of the nuptial pad was accompanied by the specially differentiated NP glands which occurred only in this area. It is still not certain, however, what kinds of substances are produced in NP gland and how this gland discharges its secretion upon what kind of stimulation. In our preliminary experiment using a pair of frogs in courtship, the nuptial pad of the male and the pieces of skin of the female biopsied during amplexus showed any noticeable histological changes. After hypophysectomy only this gland showed remarkable morphological regression of the secretory cells and this continued many days. Therefore the functional significance of NP gland is different from the mucous gland. The skin glands underneath the thumb pads in certain species have been classified as mucous glands [16–18]. Since the structural integrity of the NP glands is absolutely associated with the development of nuptial pad, this gland must have something to do with sexual behavior of the male frog, but this remains unresolved in the present experiment.

The mucous glands lost their contents enormously when the frogs were kept in waterless conditions. The contents of other kinds of glands were unchanged, although their size became small due to possible loss of water in such abnormal conditions. The skin textures gradually evolved to adapt exterior circumstances [11] and in amphibians, ions and organic substances in water passively pass through the skin [19]. The watery substance of the mucous glands may be secreted first to keep well being of an individual under the present severe experimental circumstances. In normal physiological condition it was reported that an active ion-transport rather than secretion of cellular product occurred in the secretory cells of the mucous gland, and this mechanism was not elucidated [20, 21].

Numbers of biologically active peptides and biogenic amines, similar to those of mammalians, and alkaloids have been found in amphibian skins [3, 25]. High concentrations of thyrotrophic releasing hormone (TRH) and 5-hydroxytryptamine (5-HT) in the granulated glands of *X. laevis* and *R. pipiens* have been demonstrated [22]. Dockray and Hopkins [6] and others [23, 24] demonstrated a large amount of caerulein in the granulated gland of *X. laevis*. These substances (TRH, 5-HT and caerulein) were proved to be discharged following adrenergic stimulation [6, 25]. In the present experiment we employed isoproterenol (ISP), one of the sympathomimetic substances, to observe the response of the granulated gland in normal and experimentally treated skin. Benson and Hadley [14] reported that adrenaline and noradrenaline stimulated secretion of the contents of the granulated gland but ISP was ineffective when *R. pipiens* and *X. laevis* were subjected to *in vitro* treatment with various sympathomimetic agents in concentrations of 10^{-4}, 10^{-5} and 10^{-6} M. Holmes *et al.* [15] also observed the effectiveness of adrenaline and related substances in stimulating glandular secretion of *X. laevis* skin and they ranked their stimulative effectiveness as follows; adrenaline was most effective followed by noradrenaline and phenylephrine, while isoprenaline and salbutamol of adrenergic agonist were ineffective. However, we were able to demonstrate that applications of ISP to *X. laevis* skin *in vivo* or *in vitro* caused positive responses. The secretion of a milky substance on their surfaces and changes in structures of the granulated glands occurred. Such an apparent difference between ISP effectiveness in the present series of experiments and others may not be due to the nature of the drug nor to a species specificity of the frogs employed but largely due to the concentrations of ISP applied. In the present series of experiments we applied ISP in concentrations 10^{-1} to 10^{-5} M, compared to 10^{-4} M, 10^{-5} M and 5×10^{-6} M in the experiments by Benson and Hadley [14] and 10^{-4} M and 10^{-5} M in the experiment by Holmes *et al.* [15]. Our experiment 2, in which ISP was applied to the frogs which had the detached and sewed skin pieces in their backs, the skin pieces operated on showed exceedingly retarded response to ISP in the early postoperative period followed by gradual recovery. This may be largely due to the degree of recovery of vascularization after the operation in which the vascularization of the operated skin pieces in the early phases of healing might not be sufficient to carry the amount of ISP needed for stimulation of gland secretion.

Innervations

In the present series of experiments, we also surveyed innervations of the glands studied. All the skin glands have been reported to be surrounded by myoepithelial cells either completely [26] or incompletely [27]. The innervations in the small granulated gland and the NP gland in the male nuptial pad have not been studied before. In the granulated gland and the NP gland we were able to demonstrate nerve fibers in the secretory compartments, while in the mucous gland and the small granulated gland the nerve fibers only came to have access to the glands outside the secretory compartments. Dockray and Hopkins [6] showed that nerve endings were present in the secretory compartment of the granulated gland of *X. laevis*, and Sjöberg and Flock [13] also reported a similar finding in the granulated gland of *R. temporaria* and *R. esculenta*. In mammalian species, it was also reported that similar types of innervations existed in the nasal gland in Guinea pig [28] and the lacrimal gland of sheep [29]. The present results also suggest that neural influence may be exerted in the skin glands differently, according to the individual gland, since some glands received innervation on the inside of the secretory compartments while other glands were not innervated inside the secretory compartments but were only accessed by the nerve ending structures outside the secretory compartment. The excretion of the contents of the glands may occur mainly due to contraction of the myoepithelial cells. Histological signs of contraction of the myoepithelial cells were evident through the reports by Holmes and Balls [26] and the results of the present series of experiments. However, the present study demonstrates that there were also nerve endings resting on the surfaces of the secretory cells and occasionally in a deep place far from the surface of the myoepithelial cells. Therefore it is important to study the receptor sites for the sympathomimetic substances not only through the surface of the myoepithelial cells but also of the secretory cells with thorough studies on alternations of nerve endings and the structures of the secretory cell cytoplasm, to elucidate the cellular mechanism by which the contents of the granulated gland are expelled upon nervous stimulation.

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